USE OF TREHALOSE FOR STABILIZING A LIQUID VACCINE

The invention relates to the field of vaccines. More particularly, the invention relates to liquid vaccine compositions comprising, among their antigens, at least one polysaccharide bound to a carrier protein.

Such vaccine compositions, some of whose antigens have to be bound to carrier proteins in order to be immunogenic, are known in the prior art. This is in particular the case for compositions intended for vaccination against infections caused by the bacterium Haemophilus influenzae type b, which comprise, as vaccine antigen, the capsular polysaccharide of the bacterium or Polyribosylribitol Phosphate (PRP) coupled to the tetanus toxoid T. Such vaccine compositions tend to lose their immunogenicity, and therefore their efficacy, over time. To overcome this drawback, the solution generally proposed in the prior art is freeze-drying. This solution, which is satisfactory from the point of view of the result obtained as regards preservation of immunogenicity, has, nevertheless, the disadvantage of making the method of manufacture cumbersome, and therefore of increasing the cost thereof. In addition, at the time of administration of the vaccine, it is necessary to carry out an additional operation of taking up the freeze-dried product in a sterile liquid, which, on the one hand, represents an additional constraint for the practitioner and, on the other hand, comprises, like any manipulation, the risk of being poorly carried out. It is therefore desirable to find another solution to the problem of the loss of immunogenicity, over time, of the polysaccharide antigens bound to a carrier protein when they are present in a liquid vaccine composition.

To this end, the invention provides a liquid vaccine composition comprising at least one antigen consisting

of a polysaccharide bound to a carrier protein, characterized in that it additionally comprises trehalose.

Thus, a vaccine composition is obtained which, although liquid, preserves it immunogenic character over time, even when it is stored at room temperature.

The subject of the present invention is also a method of stabilizing a liquid vaccine composition comprising at least one antigen consisting of a polysaccharide bound to a carrier protein, characterized in that it consists in adding trehalose to the vaccine composition.

The method according to the invention has the advantage of being simple and fast, which makes it a method of choice for a manufacturer.

Numerous other advantages of the present invention will emerge on reading the detailed description which follows.

The vaccine composition according to the invention may be a monovalent composition, that is to say that it is intended for protection against a single disease, or a multivalent composition, that is to say that it is intended to protect the individual to whom it has been administered, against several diseases. In all cases, at least one of the vaccine valencies is represented by a polysaccharide antigen bound to a carrier protein. Among the polysaccharide antigens capable of entering into the composition of a vaccine and of being stabilized according to the invention, there may be mentioned the polysaccharides present in the capsules of bacteria, the polysaccharides present in the walls of Gram-negative bacteria or the polysaccharides present in the walls of fungi. Thus, it is possible to use the polysaccharides encountered in the following

microorganisms: Pseudomonas (for example P. aeruginosa), Saphylococcus, Steptococcus (for example S. pneumoniae), Klebsiella (for example K. pneumonia), Salmonella (for example S. typhi and S. paratyphi), Escherichia coli, Neisseria (for example N. meningitidis), Shigella (for example S. dysenteria, somnei or flexneri), Haemophilus (for example H. influenzae type b), Moraxella, Vibrio cholerae, Mycobacterium tuberculosis, Candida, Cryptococcus neoformans and Hansenula.

The present invention has shown all its benefit for vaccine compositions comprising the capsular polysaccharide of Haemophilus influenzae type b or Polyribosylribitol Phosphate.

The polysaccharides generally used as vaccine antigens generally exhibit the characteristic of being T-independent, that is to say in particular that the memory of the immune system in relation to such antigens is weak and that these polysaccharides are generally not immunogenic in young children. To make them T-dependent, it is customary to combine them with carrier proteins (protein, for the purposes of the present invention, also includes peptides or polypeptides) in order to obtain a polysaccharide-carrier protein conjugate. These proteins are in particular those normally used in the field of vaccines: diphtheria toxoid, tetanus toxoid, nontoxic mutant form CRM_{197} of diphtheria toxoid, outer membrane protein type 1 (OMP1) of Neisseria meningitidis, as well as any native or synthetic peptide or polypeptide capable of fulfilling the same function, for example the peptides described in patent application WO98/31393.

The binding of the polysaccharide to the carrier protein can vary according to the polysaccharide and the protein used. It generally involves covalent

bonding, which can call into play a spacer arm. According to the mode of binding used, the antigen obtained, which is generally called conjugate, is an antigen in which the polysaccharide is bound to the carrier protein by a single chemical functional group (conjugates of the sun or neoglycoconjugate type) or by several functional groups (conjugates of the scraper or coil type).

As the vaccine composition according to the invention may be multivalent, it is possible to add to the antigen consisting of the polysaccharide-carrier protein conjugate one or more other valencies also consisting of a polysaccharide-carrier protein conjugate, or of any other different type of antigen. Among the other valencies which may enter into the vaccine composition according to the invention, there may be mentioned in particular: whooping cough, polio, diphtheria, tetanus, hepatitis (A, B, C and the like), varicella, mumps, measles, Dengue, Japanese encephalitis, yellow fever, rubella, influenza, meningitis, pneumonia, and the like.

The vaccine composition according to the invention may comprise, in addition, all the components usually present in a vaccine: buffer or physiological saline, preservative and one or more adjuvants.

According to a characteristic of the invention, this vaccine composition comprises, in addition, trehalose in a sufficient quantity to allow the immunogenicity of the antigen consisting of the polysaccharide conjugate to be maintained over time.

Trehalose or α-D-glucopyranosyl α-D-glucopyranoside is a disaccharide known for its protective action in relation to proteins when they are subjected to high temperatures, in particular during drying or freeze-drying operations. According to the teaching of

document US 4 891 319, its protective action may be explained by a replacement of the water molecules by trehalose molecules, the 2 compounds comprising OH functional groups.

Trehalose is also known in the prior art as a cell protectant.

Surprisingly, and without this being deducible from the known properties of trehalose, it has now been found that this compound makes it possible to preserve the immunogenicity of vaccine compositions, even in the case where the latter might not be subjected to a rise in temperature or to a drying process.

On the other hand, other sugars tested which are known to have properties similar to trehalose, in particular lactose, did not lead to satisfactory results.

According to a particular characteristic of the invention, it has been observed that a quantity of trehalose of between 3 and 12%, and preferably 5, was satisfactory to solve the problem of stability of the vaccine composition. At this concentration, no toxicity reaction was revealed.

The trehalose suitable for the purposes of the invention should be a trehalose of pharmaceutical quality, without it being necessary, nevertheless, for it to have an absolute degree of purity. The trehalose provided by the company SIGMA under the reference T9531 is perfectly suitable.

The trehalose may be added at the beginning of the method of manufacture; it may also be added to the formulation at the end of the method, alone or in the form of a mixture with other excipients.

The following examples illustrate more particularly one embodiment of the invention.

Example 1

Three different vaccine compositions are prepared by proceeding in the following manner:

a- Manufacture of 4 stock solutions of excipients

- 50 mM solution of Tris Hydroxyl Amino Methane - 42.5% sucrose

Composition for 1 liter:
6.06 g Tris Hydroxyl Amino Methane
425 g sucrose
Water for injection qs 1 liter

- Solution of trehalose at 20%

Composition for 400 ml
Trehalose: 80 g
Water for injection qs 400 ml
pH = 7 ± 0.1 after adjusting with a 2.5N sodium hydroxide solution

- 2M solution of sodium chloride

Composition for 1 liter
Sodium chloride: 117 g
Water for injection qs 1 liter

- 50 mM Tris Hydroxyl Amino Methane

Composition for 1 liter
Tris Hydroxyl Amino Methane: 6.06 g
5N HCl: 8.54 ml
Water for injection qs 1 liter

b- Production of 3 solutions of excipients (A, B, C) from the preceding 4 stock solutions. The volumes of the stock solutions used are indicated in the table below:

Stock solutions	Excipient 1	Excipient 2	Excipient 3
50 mM Tris Hydroxyl Amino Methane solution, 42.5% sucrose	72.6 ml	0	0
20% trehalose solution	0	100 ml	200 ml
2M sodium chloride solution	0	19 ml	0
50 mM Tris Hydroxyl Amino Methane solution	0	72.6 ml	72.6 ml
Water for injection	qs 363 ml	qs 363 ml	qs 363 ml

c- Each solution of excipient is sterilized by filtration on a Millipack 60 filter having a cut-off of 0.22 µm.

d- To obtain each of the vaccine compositions, there are added to a 500 ml Schott flask, sterilized by autoclaving, in the order: 290 ml of each of the solutions of excipients prepared and then 29.6 ml of a composition containing PRP-T and sucrose. The flasks are stirred for 5 minutes at room temperature and then for 2 hours at 4°C.

e- Each composition is distributed into glass serum bottles which are kept for 6 months at 25°C.

The final composition of each formulation is summarized in the following table:

	Composition A	Composition B	Composition C`
PRP-T (µg of polysaccharide/ml)	20	20	20
Tris Hydroxyl Amino Methane (mM)	10 mM	10 mM	10 mM
Sucrose (%)	8.5%	0.78%	0.78%
Trehalose (%)	0%	5%	10%
NaCl (mM)	0	0.095 mM	0

Example 2

Five groups of 8 female OF 1 mice, weighing 22 to 24 grams, are available. The mice are divided into groups of 8. Each group is used to test one of the vaccine compositions A, B or C obtained in example 1, a vaccine composition serving as negative control (comprising only nonconjugated PRP) and a vaccine composition serving as positive control which consists of the vaccine Act-Hib™ marketed by the company PASTEUR MERIEUX Sérum and Vaccins.

0.5 ml of the vaccine composition to be tested, corresponding to 2.5 µg of polysaccharide, is administered to each mouse, by the subcutaneous route. Each mouse receives one injection at D0 and one booster injection at D14.

The serum of each mouse is collected at D0, D14 and D21.

Example 3

The sera collected are assayed by RadioImmunoAssay. The results obtained are exploited in the following manner:

- The geometric mean is calculated from the titer of 8 sera.
- The % of responsive mice (serum having a titer > 0.5) is determined.
- The difference between the titers obtained at D14 and D21 is calculated so as to evaluate the effect of the booster injection.

The product is declared to be in conformity when the following 3 conditions are met:

- At D21, at least 75% of the mice have a titer >=0.5.
- The difference between the titers obtained at D14 and D21 is statistically significant.

- The difference in titer between the product tested and the positive control is not statistically significant at D21.

The results obtained are summarized in the following table:

	GMT at D14	**GMT at D21**	**Conformity of the product**
Negative control	< 0.09	0.05	-
Positive control	< 0.1	1.3	+
Composition A	0.33	0.99	-
Composition B	0.13	1.6	+
Composition C	0.11	2.9	+

These results show that the vaccine compositions according to the invention preserve their immunogenicity after storage for 6 months at 25°C.

Tests carried out in the same manner on compositions stored at 37°C showed that a composition according to the invention, comprising 5% trehalose, retained its immunogenic character even after 3 months of storage.